18. Roy, S., Wyse, B., and Hancock, J.F. (2002). H-Ras signaling and K-Ras signaling are differentially dependent on endocytosis. Mol. Cell. Biol. *22*, 5128–5140.
19. Jura, N., Scotto-Lavino, E., Sobczyk, A., and Bar-Sagi, D. (2006). Differential modification of Ras proteins by ubiquitination. Mol. Cell *21*, 679–687.
20. Galperin, E., and Sorkin, A. (2008). Endosomal targeting of MEK2 requires RAF, MEK kinase activity and clathrin-dependent endocytosis. Traffic *9*, 1776–1790.

NYU Cancer Institute, 550 First Avenue, New York, NY 10016, USA.
*E-mail: philim01@med.nyu.edu

DOI: 10.1016/j.cub.2009.04.045

Gut Microbiology: Fitting into the Intestinal Neighbourhood

Microbes inhabiting the gut affect our health in profound and unexpected ways: new studies now show that these effects depend on synergistic and competitive interactions between the bacteria, which are influenced by diet.

B.P. Willing[1] and B.B. Finlay[1,2,3]

Our gastrointestinal tracts teem with an immensely abundant and diverse microbial population. That we have a symbiotic relationship with this population is evident as resident bacteria increase our digestive capabilities, create nutrients that would otherwise not be present and exclude harmful pathogens. There is, however, a growing appreciation for how intertwined our physiology is with that of our inhabitants [1]. Gut bacteria have recently been found to impact many aspects of human health, including immune development, inflammatory bowel diseases, obesity, diabetes, allergic diseases, enteric diseases and cancer [2–5]. Now that studies have revealed a diverse set of microbial products that can be detected systemically within the host, these effects are not so surprising [6].

Each individual's digestive tract is home to a conservative estimate of 500–1000 bacterial species, most of which cannot currently be cultured, and whose genomes collectively represent approximately 100 times the number of genes present in the human genome [7]. There is large inter-individual variation in community composition at the species level; however, the microbial composition of all healthy individuals is dominated by two main phyla, Bacteroidetes and Firmicutes [8].

Some diseases, such as Crohn's, are linked to the disappearance or appearance of specific members of these phyla [2], while others, such as obesity, are linked to a more general shift in the ratio of Bacteroidetes to Firmicutes [3]. However, the causes of these population shifts are not apparent, nor are the mechanisms by which these shifts result in disease or susceptibility. It has therefore become apparent that understanding the mechanisms that govern the bacterial population and how they exert their effects on each other and on the host will be essential to facilitate the development of strategies to manipulate the microbiota to promote health.

Metagenomic and metaproteomic approaches have made it possible to broadly explore the biological processes driving this complex community. For example, a surprising proportion of proteins found in the faeces were identified as ones involved in innate immune defense, indicating an extensive effort of the host to regulate the microbial population [9]. But these methods are highly dependent on computational analysis of DNA or protein sequences, and because of the diversity of the microbiota, interactions between these systems are not easily pieced together.

A recent study by Mahowald *et al.* [10] begins to unravel the complexity of the relationships that govern the community ecology of the gastrointestinal tract by creating and characterizing a highly simplified model gut microbiota. *Bacteroides thetaiotaomicron* and *Eubacterium rectale*, chosen as representative organisms of the two main phyla, Bacteroidetes and Firmicutes, respectively, were introduced into 'gnotobiotic' mice, which initially lack any gut bacteria. The mice were either colonized with each bacterium alone (mono-association) or co-colonized by both species, and then transcriptional profiling of bacteria and host was performed.

Using this model system, Mahowald *et al.* [10] demonstrate a number of interactions that have previously been hypothesized but have not been shown *in vivo* (Figure 1). Firstly, they show that these two bacteria change their behaviour as a result of competition for nutrients. *B. thetaiotaomicron* responds to co-colonization by increasing its repertoire of glycan-degrading enzymes and signaling the host to produce glycans that it, but not *E. rectale*, can utilize. Comparison of the genomes of *B. thetaiotaomicron* and other sequenced Bacteroidetes to those of *E. rectale* and other sequenced Firmicutes revealed that Bacteroidetes contain a relative surplus of glycan degrading enzymes. This may suggest that foraging on host glycans as a result of competition with Firmicutes may be a common adaptation used to remain competitive. Conversely, *E. rectale* appears to more effectively access nutrients in the presence of *B. thetaiotaomicron*, as evidenced by increased expression of a number of amino acid and peptide transporters.

The results of this study also indicate there are synergistic interactions between these two bacteria. It appears that acetylCoA produced by *B. thetaiotaomicron* is utilized by *E. rectale* and is subsequently converted to butyrate. Crossfeeding between gut bacteria has been observed before *in vitro* [11], but this is the first time that synergistic actions between bacteria have been shown to occur *in vivo* and to impact on host physiology, as many expression changes observed in co-colonized mice have been reported to be affected by increased butyrate production [12]. Previous studies of how the host responds to single bacterial species have shown that each species can affect the expression of hundreds of host genes [13], and that different commensal organisms have dramatically different effects [14]. Important to the interpretation of host–microbe interactions, the Mahowald *et al.*

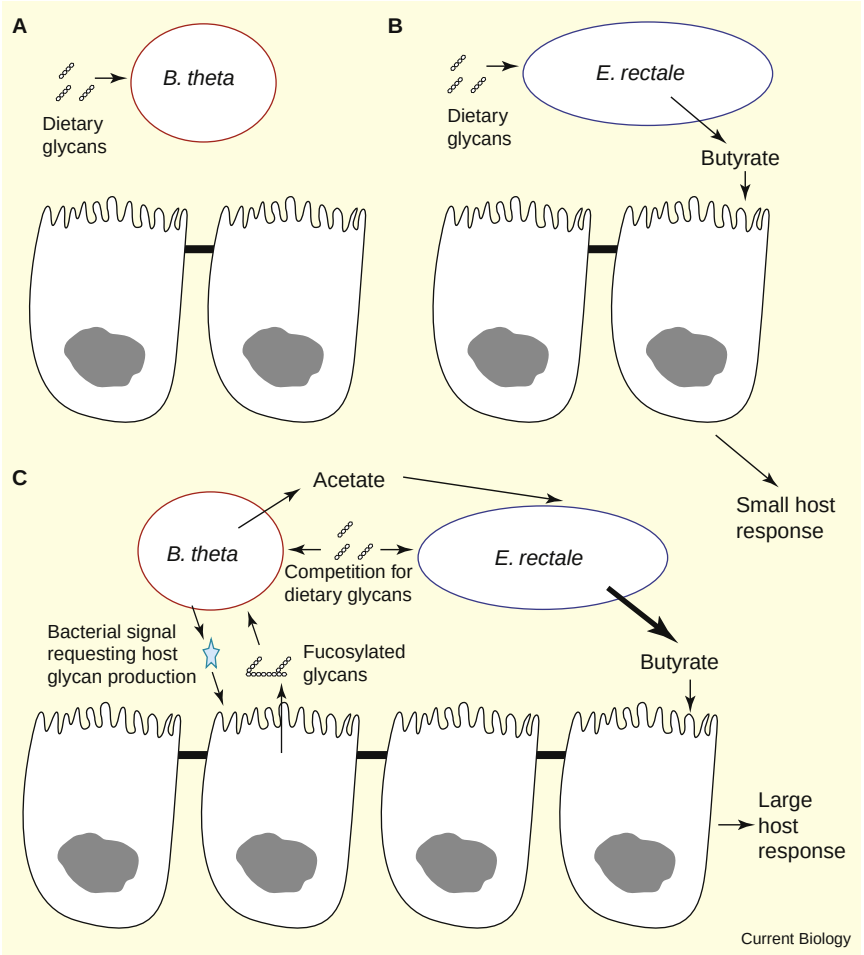

Figure 1. Microbe–microbe and microbe–host interactions in the gut.

Summary of microbe–microbe and microbe–host interactions between *Bacteroides thetaiotaomicron* (*B. theta*) and *Eubacterium rectale* (*E. rectale*) in the gnotobiotic mouse model with: (A) *B. theta* in mono-association; (B) *E. rectale* in mono-association; and (C) *B. theta* and *E. rectale* in di-association. *B. theta* responds to *E. rectale* by signaling the host to produce fucosylated glycans that it, but not *E. rectale,* can degrade. Production of acetate by *B. theta* is utilized by *E. rectale* to produce increased levels of butyrate, resulting in amplified host responses.

study [10] indicates that the way that bacteria affect the host is dependent on interactions with other bacteria. It cannot therefore be assumed that the host is affected in a given way based on the presence of a single organism and that multiple interactions must be taken into account.

As well as examining how microbe–microbe interactions affected host physiology, Mahowald *et al.* [10] looked at how changes in diet affect interactions between the bacteria. By comparing the effects of a diet low in fat and high in plant polysaccharides (a healthy diet) to those of one high in fat and with low plant polysaccharides (a typical unhealthy/Western diet) in mice colonized with both *E. rectale* and *B. thetaiotaomicron*, they found that *B. thetaiotaomicron* colonization was unaffected by diet, whereas *E. rectale* colonization was reduced significantly by the removal of plant polysaccharides. Reduced *E. rectale* number was also associated with reduced butyrate production, indicating the functional significance of changes in the diet on intestinal health, as butyrate is the preferred energy source for the colonic epithelium [15].

This study demonstrates that bacterial signaling to the host is modified as a result of changes in the intestinal environment created by neighbouring bacteria and/or diet. The fact that changes in the composition of the microbiota result in increased susceptibility to *Salmonella enterica* infection may suggest a similar interaction [16]. Some recent evidence suggests that changes in the luminal environment resulting from bacterial fermentation do indeed impact virulence regulation [17].

Complicating the system even more, the host also plays an important role in the competition between bacteria. *B. thetaiotaomicron* (Gram-negative) stimulates host expression of genes responsible for antimicrobial activity targeting Gram-positive bacteria, while *Bifidobacterium longum* (Gram-positive) suppresses the expression of these same genes [18]. Another interaction through the host immune system is indicated by a mechanism where the normal flora protects the host from *Enterococcus* infection by inducing host expression of a c-type lectin that kills Gram-positive bacteria [19]. These interactions are indirect and require a functional immune system. Because how the host responds to microbes largely depends on host immunity, and it is apparent that the immune function of the gnotobiotic animal is drastically different from that of a conventional animal, indirect interactions between bacteria that are dependent on a functional immune system may be missed in the simplified model system reported by Mahowald *et al.* [10].

It is yet unclear whether these two organisms have evolved to act in concert, or are simply responding to the environment created by the other organism. Whether the interactions observed here between *E. rectale* and *B. thetaiotaomicron* are representative of a common interaction between Bacteroidetes and Firmicutes require further validation. The extent to which expression patterns are changed in both bacteria and host identify how complicated the entire community must be, with over 100,000 possible direct microbe–microbe interactions (assuming a modest estimate of 500 bacterial species).

References

1. Martin, F.J., Sprenger, N., Yap, I.K., Wang, Y., Bibiloni, R., Rochat, F., Rezzi, S., Cherbut, C., Kochhar, S., Lindon, J.C., *et al.* (2009). Panorganismal gut microbiome-host metabolic crosstalk. J. Prot. Res. *8*, 2090–2105.
2. Willing, B., Halfvarson, J., Dicksved, J., Rosenquist, M., Jarnerot, G., Engstrand, L., Tysk, C., and Jansson, J.K. (2008). Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. Inflamm. Bowel. Dis. *15*, 653–660.
3. Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I.

(2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature *444*, 1027–1031.
4. Wen, L., Ley, R.E., Volchkov, P.Y., Stranges, B., Avanesyan, L., Stonebraker, A.C., Hu, C., Wong, F.S., Szot, G.L., Bluestone, J.A., *et al*. (2008). Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature *455*, 1109–1113.
5. Rakoff-Nahoum, S., and Medzhitov, R. (2007). Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. Science *317*, 124–127.
6. Wikoff, W.R., Anfora, A.T., Liu, J., Schulta, P.G., Lesley, S.A., Peters, E.C., and Siuzdak, G. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc. Natl. Acad. Sci. USA *106*, 3698–3703.
7. Hooper, L.V., and Gordon, J. (2001). Commensal host-bacterial relationships in the gut. Science *292*, 1115–1118.
8. Turnbaugh, P., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., *et al*. (2009). A core gut microbiome in obese and lean twins. Nature *457*, 480–485.
9. Verberkmoes, N.C., Russel, A.L., Shah, M., Godzik, A., Rosenquist, M., Halfvarson, J., Lefsrud, M.G., Apajalahti, J., Tysk, C., Hettich, R., *et al*. (2009). Shotgun metaproteomics of the human distal gut microbiota. ISME J. *3*, 179–189.
10. Mahowald, M.A., Rey, F.E., Seedorf, H., Turnbaugh, P.J., Fulton, R.S., Wollam, A., Shah, N., Want, C., Magrini, V., Wilson, R.K., *et al*. (2009). Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. Proc. Natl. Acad. Sci. USA *106*, 5859–5864.
11. Belenguer, A., Duncan, S.H., Calder, A.G., Holtrop, G., Louis, P., Lobley, G.E., and Flint, H.J. (2006). Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. Appl. Environ. Microbiol. *72*, 3593–3599.
12. Daly, K., and Shirazi-Beechey, S.P. (2006). Microaray analysis of butyrate regulated genes in colonic epithelial cells. DNA Cell Biol. *25*, 49–62.
13. Hooper, L., Wong, M., Thelin, A., Hansson, L., Falk, P., and Gordon, J. (2001). Molecular analysis of commensal host-microbial relationships in the intestine. Science *291*, 881–884.
14. Willing, B.P., and Van Kessel, A.G. (2007). Enterocyte proliferation and apoptosis in the caudal small intestine is influenced by the composition of colonizing commensal bacteria in the neonatal gnotobiotic pig. J. Anim. Sci. *85*, 3256–3266.
15. Roediger, W.E. (1982). Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterol. *83*, 424–429.
16. Sekirov, I., Tam, N.M., Jogova, M., Roberson, M.L., Li, Y., Lupp, C., and Finlay, B.B. (2008). Antibiotic-induced perturbations of the intestinal microbiota alter host susceptibility to enteric infection. Infect. Immun. *76*, 4726–4736.
17. Carey, C.M., Kostrzynska, M., Ojha, S., and Thompson, S. (2008). The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic *Escherichia coli* O157:H7. J. Microbiol. Methods *73*, 125–132.
18. Sonnenburg, J.L., Chen, C.T.L., and Gordon, J.I. (2006). Genomic and metabolomic studies of the impact of probiotics on a model gut symbiont and host. PLoS Biol. *12*, 2213–2226.
19. Brandl, K., Plitas, G., Mihu, C.N., Ubeda, C., Jia, T., Fleisher, M., Schnabl, B., DeMatteo, R.P., and Pamer, E.G. (2008). Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature *455*, 804–807.

[1]Michael Smith Laboratories, [2]Department of Microbiology and Immunology, and [3]Department of Biochemistry and Molecular Biology, University of British Columbia, #301-2185 East Mall, Michael Smith Building, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.
E-mail: bfinlay@interchange.ubc.ca

DOI: 10.1016/j.cub.2009.04.025

Meristems: The Root of Stem Cell Regulation

The stem cells from which the plant body develops are located in shoot and root meristems, and new research shows that the balance between proliferation and differentiation in each is controlled by related proteins that interact in a similar feedback network.

Liam Dolan

Plant bodies are made up of repeating units that are produced throughout the life of the plant from multiple populations of stem cells surrounded by rapidly dividing cells in meristems.

A network with feedback controls the balance between proliferation and differentiation in the shoot meristem [1,2]. The proliferation of stem cells is negatively controlled by the small peptide CLV3 that accumulates in the stem cells. Loss of CLV3 activity in mutants results in the loss of this negative regulation, resulting in increased stem-cell proliferation compared with wild-type plants.

CLV3 transcription is positively regulated by the WUSCHEL (WUS) protein, a homeobox transcription factor that is expressed in cells positioned just below the stem cells, a region that is often called an 'organiser' because it controls the proliferative activity of the overlying stem cells. CLV3, in turn, negatively regulates *WUS* transcription. In this way, a negative feedback loop controls the balance between proliferation and differentiation of stem cells in the shoot meristem.

In the root, the cells of the quiescent centre — a group of approximately four cells that are mitotically quiescent and signal to surrounding cells, maintaining them in a stem-cell state — also act as an organising centre. Laser ablation experiments showed that the quiescent centre signals to the surrounding cells, maintaining them in a stem-cell state and inhibiting their differentiation [3]. Furthermore, a CLV3-like protein called CLE40 negatively regulates stem-cell function in the root, while a WUS-related protein called WOX5 positively regulates stem-cell development [4].

Despite these parallels, the precise nature of the interaction between CLE40 and WOX5 was unclear. Now, Stahl *et al.* [5], in this issue of *Current Biology*, show that WOX5 positively regulates the production of CLE40 protein. CLE40, in turn, negatively regulates *WOX5* transcription. This means that CLE40 and WOX5 form a self-regulating network in the root that, much like the CLV3–WUS network in the shoot, controls the proliferation and differentiation of stem cells.

Stahl and co-workers [5] show that loss of WOX5 activity results in a phenotype that is similar to that of plants with excess levels of CLE40 — more stem cells differentiate as root cap and, consequently, there are fewer stem cells than in wild-type plants. In contrast, the increase in the number of stem cells and a reduction in the number of differentiated root cap cells in plants that overexpress *WOX5* is similar to the phenotype of plants that are homozygous for *cle40* loss-of-function alleles. These phenotypes indicate that CLE40 and WOX5 play opposite roles in the differentiation of stem cells in the distal root meristem.

The opposite roles played by CLE40 and WOX5 suggest that they might regulate each other, as previously demonstrated for the paralogous proteins in the shoot meristem. Indeed,